EVALUATION OF GENE EXPRESSION AND CYTOGENETICS ANOMALIES FROM PERIPHERAL BLOOD AND HISTOPATHOLOGICAL VARIATIONS IN TESTIS OF MALE TO FEMALE TRANSGENDERS (MTF) UNDERGOING SEX REASSIGNMENT SURGERY.

Synopsis

Faculty of
Multidisciplinary Health Sciences and Technology

Submitted to

SRI BALAJI VIDYAPEETH

(Deemed to be University)
Accredited by NAAC with A++ Grade
Pondicherry - 607402
Submitted by

Mrs. Preetha Devi N B
(UIN: 2005003002)

Under the guidance of

Dr. Uma A N
Professor of Medical Genetics
Principal, School of Allied Health Sciences
SRI BALAJI VIDYAPEETH
(Deemed to be University)
Pillaiyarkuppam, Pondicherry - 607402, India.

June, 2024

CERTIFICATE

This is to certify that the synopsis of the thesis titled "**Evaluation Of Gene Expression And Cytogenetics Anomalies From Peripheral Blood And Histopathological Variations In Testis Of Male To Female Transgenders (MtF) Undergoing Sex Reassignment Surgery.**" submitted for the Degree of Doctor of Philosophy in Multidisciplinary Health Sciences and Technology, Sri Balaji Vidyapeeth is a bonafide work done by **Mrs. Preetha Devi N B, UIN. No. 2005003002, Faculty of Multidisciplinary Health Sciences and Technology, Medical Genetics, Mahatma Gandhi Medical College & Research Institute, Puducherry – 607402, during the period from July 2020 – June 2024 under my guidance and supervision. This research work is original and has not been submitted elsewhere for the award of any other Degree or Diploma or Associateship or Fellowship or any other similar titles.**

A.N Uma

Dr. Uma A N
Supervisor and Guide

Date: 28.06.2024

Place: Puducherry

Declaration

I hereby declare that the synopsis of the thesis titled "**Evaluation of Gene Expression And Cytogenetics Anomalies From Peripheral Blood And Histopathological Variations In Testis of Male To Female Transgenders (MtF) Undergoing Sex Reassignment Surgery"** submitted for the Degree of Doctor of Philosophy in the faculty of Multidisciplinary Health Sciences and Technology, Sri Balaji Vidyapeeth is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associate ship or fellowship of similar other titles. It has not been submitted to any other university or institution for the award of any degree or diploma.

Mrs. Preetha Devi. N. B
PhD scholar

Date: 28.06.2024

Place: Puducherry

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INTRODUCTION

Transgender people are people who identify with a gender that is different from their genetic sex and also from their physical sex, but who are actively transitioning their physical form, social position, and legal status to fit their gender identity. The term "Gender Incongruence" (GI) refers to a condition in which a person does not identify with the gender assigned to them at birth based on physical sexual traits, resulting in "a noticeable and persistent incongruence between the experienced gender and the assigned sex.

GENDER DYSPHORIA AND GENDER AFFIRMATION SURGERY IN TRANSGENDERS

Gender dysphoria (GD) occurs when people with gender incongruence experience clinically meaningful biopsychosocial pain, according to the DSM-5 classification. Physical transition is the greatest choice for many trans people to alleviate the symptoms of gender dysphoria. Sex/gender reassignment hormone therapy, as well as surgery, play important roles in this scenario. The latter include genital surgery (sex reassignment surgery) (Box), breast surgery, face and voice chord surgery, and hair epilation [1].

The most severe manifestation of this disease is transsexualism. These people believe they are locked in the wrong body (transsexual phenomenon). They must modify their phenotypic with hormones and surgery to match their gender identification. Gender Affirmation Surgery may be the most acceptable word (GAS) for transsexuals undertaking Female to Male (FTM) transition, referred to as transmen, whereas transsexuals undergoing Male to Female (MTF) transition are referred to as transwomen [2].

This surgery has been demonstrated to be usually beneficial, and the suicide incidence among transsexuals appears to be significantly lower. Cross Sex Hormone Therapy (CSHT) aims to help patients achieve and maintain these bodily changes.

CROSS HORMONE THERAPY AND CYTOGENETICS STUDY IN TRANSGENDERS

Cytogenetic study on transgenders as part of a medical evaluation for GD may be useful for other reasons. Karyotyping is used to detect the biological gender of most professionals in many countries during the transition phase before hormone therapy as sometimes they may have sex chromosomal variations. We need to understand that feminization refers to all of the physical changes that allow transgender women (i.e., MtF people) to live in society as women, including reduced body hair covering, breast growth, skin softening, and gynoid fat distribution. Feminization of transgender women is accomplished by the injection of oestrogen in conjunction with an antiandrogen medicine to reduce testosterone and lessen masculine physical traits [3].

Oral, transdermal, or injectable 17-estradiol is the most often used oestrogen, whereas cyproterone (mostly used in Europe) and spironolactone (primarily used in the United States) are preferred as antiandrogens over flutamide or gonadotropin releasing hormone agonists [4] Yet all transgenders fail to realise that such cross-hormone therapy due to toxicity of the medication, could lead to adverse ill-health, chromosomal aberrations in the peripheral blood leading to instability of chromosomes during cell division, and nuclear damage as identified from the buccal cells leading to oral cancer [5]

CROSS HORMONE THERAPY AND HISTOPATHOLOGY OF TRANSGENDER TESTIS AFTER GENDER AFFIRMATION SURGERY

A couple of studies have been reported that suppression of puberty with gonadotropin-releasing hormone agonist analogs (GnRHa) in the paediatric transgender patient can pause the maturation of germ cells, and thus, affect fertility potential. Oestrogen therapy in transgender women has been found to lead to impaired spermatogenesis and testicular atrophy leading to

infertility which has been well documented by histopathological studies on testis of transwomen who had undergone Gender-affirming surgery (GAS) [6] Addressing the importance on fertility preservation in order to recognize gender affirming treatment impact on transgender health seems to be the need of the hour [7] .

EVIDENCE FOR GENE VARIANTS IN TRANSGENDERS

More recently, particular genes have been studied in detail in transwomen and transmen. One study looked at associations between being trans and particular variants of some genes in the hormone pathway. A recent and much larger study assembled samples from 380 transwomen who had, or planned, sex change operations. They looked in fine detail at 12 of the "usual suspects" – genes involved in hormone pathways. They found that transwomen had a high frequency of particular DNA variants of four genes that would alter sex hormone signalling while they had been developing in utero. There may be many other genes that contribute to a feminine or a masculine sexual identity. They are not necessarily all concerned with sex hormone signalling – some may affect brain function and behaviour. The next step in exploring this further would be to compare whole genome sequences of cis- and transsexual people. Whole genome epigenetic analyses, looking at the molecules that affect how genes function in the body, might also pick up differences in the action of genes. It's probable that many – maybe hundreds – of genes work together to produce a great range of sexual identities [8] Also an argument by Zicklin, 1997, has described the so-called "gay genes", suggesting same–sex attraction can readily be explained by many "male-loving" and "female-loving" variants of mate choice genes that seems to be inherited independent of sex.

GENDER DYSPHORIA AND MUTATIONS IN RYR3

Gender dysphoria (GD), a contentious diagnostic category in the American Psychiatric Association's (APA) Diagnostic Statistical Manual of Mental Disorders (DSM) 5, is characterised by persistent dissatisfaction with one's biologic sex. It is believed that GD affects

less than 1 in 10,000 adult natal men and 1 in 30,000 adult natal females. The aetiology of GD is thought to be multifaceted, including both biological (hormonal and genetic) and psychological factors [9].

Gender identity is thought to be anatomically located in the brain[8]. Some regions in the human brain are bigger in men (such as the hypothalamus and amygdala) and smaller in females (such as the caudate nucleus and hippocampus) [10].

However, it is unknown what function these dimorphisms have in the development of GD. Mutations in RYR3 may result in an imbalance in intracellular calcium homeostasis, impairing neuronal function.

This gene encodes a ryanodine receptor, which acts to release calcium from intracellular storage for usage in a variety of cellular activities. The encoded protein, for example, is engaged in skeletal muscle contraction by releasing calcium from the sarcoplasmic reticulum and then depolarizing T-tubules. This gene has two transcript variants that encode distinct isoforms. While single ion transporter genes, such as RYR3, may contribute to GD risk, the cumulative influence of ion transporter gene variations as a class on GD susceptibility is as apparent. The majority of genes in this category may contribute to GD risk, although the risk may also grow proportionately with an individual's number of variations. [10]

Since the Department of General Surgery, Mahatma Gandhi Medical College & RI, Pondicherry, performs male to female Sex Reassignment Surgery, the histopathological studies on their testis, chromosomal study (cytogenetic assay) and molecular study (Gene expression) from their peripheral blood was conducted meticulously to see if the findings may pave way to some convincing answers to questions still not answered for the cause of the transgender community at large

REVIEW OF LITERATURE

It is well recognized that both biological and environmental factors have an impact on the intricate process of sexual differentiation. The goal of this study is to provide an overview of the most pertinent research on the biological underpinnings of sexual development. Specifically, it focuses on the effects of sex hormones and genetic background on the maturation of gender identity and sexual differentiation. According to the research, gonadal hormones may shape psychosexual development during the foetal and pubertal stages, with the sexually dimorphic brain serving as the structural basis. Furthermore, a number of heritability studies suggest that genetic factors could be involved, however a viable candidate gene has not yet been found. Even while there is mounting evidence that biological variables play a fundamental role in psychosexual development, more research is required to fully understand the intricate interplay between these elements (Hyde et al., 2008).

The process of sexual differentiation is the evolution of distinctions between males and females, which affect people as well as being often seen in nature (Zosuls et al., 2011). Gender identity, defined as the inner feeling of self as a female, a male, or as an alternate gender, different from the male and female ones, is one of the most sexually dimorphic characteristics of humans. Gender identity develops in cisgender people in accordance with their given gender at birth and remains constant throughout life. However, transgender people may identify either temporarily or permanently with a gender other than the one they were given at birth. Gender dysphoria is defined as the incongruence between the experienced/expressed gender and the assigned one that results in clinically significant psychological distress and impairment in the major areas of functioning, according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM 5). A desire for a social and/or physical transition through

gender affirming hormonal therapy and surgery may arise in certain circumstances due to this discomfort (Dora et al., 2021).

Understanding the genesis of the sexual differentiation process has received a lot of research due to its complicated framework and therapeutic implications. The role of nature vs nurture in the sexual differentiation of the brain is a topic of much discussion in the literature. Nevertheless, the importance of biology is well acknowledged. Indeed, studies on the neuroanatomy and sexual dimorphism of the brain in recent years have mostly examined the impact and determining function of several genes and sex hormones (Ristori et al., 2020). Specifically, it is thought that the sexually dimorphic brain serves as the structural foundation for psychosexual development, and that gonadal hormones and genes may shape this development. Prenatal and pubertal sex hormones have been shown to have a lasting impact on human conduct, and heritability studies have indicated the contribution of genetic factors. In fact, there are physical variations between cismen and ciswomen in the overall volume of the brain and in a number of sex-dimorphic structures. In instance, transgender men's brain sizes were shown to be comparable to their assigned gender at birth, while the overall brain volume is larger in cismen. Nonetheless, transwomen's total cerebral capacity was found to be halfway between that of male and female controls. Additionally, in a number of locations, ciswomen have been found to have a larger cortical thickness than cismen. Research on transgender people revealed that transwomen showed symptoms of feminization in their cortical thickness, whereas transmen showed no signs of masculinization (Zubiaurre-Elorza et al., 2014).

The hippocampus and amygdala appear to represent another region of sexual dimorphism. In fact, parts of the hippocampus are bigger in women and have a greater density of oestrogen than androgen receptors (AR), whereas the amygdala is larger in males and has a higher density

of androgen than oestrogen receptors. These variations beg the question of whether cross-gender identifications are a reflection of the structure and/or function of the brain. Because of this, a growing body of research is concentrating on the structural and functional differences between transgender and cisgender people in various brain regions, particularly in those who exhibit sexual dimorphism, employing both post-mortem and in vivo neuroimaging investigations (Kight et al., 2020).

AIM AND OBJECTIVES

Most authors consider GD to have multifactorial origins: genetic, biological, psychological and social. The increased incidence of GD in the context of Differences/Disorders of Sexual Development (DSD) has led to the hypothesis that sex steroids and substance abuse play a role in the etiology of GD, and that mutations in the genes encoding enzymes involved in their biosynthesis or metabolism or encoding their receptors could be implicated.

The main goal of our research is to assess and analyze the gene expression and Cytogenetic anomalies found in peripheral blood and the histopathological variation observed in the testis of Male to Female Transgender undergoing Sex Reassignment Surgery.

OBJECTIVES OF THE STUDY

- The studies were conducted in transgender individuals without associated GD, versus same-sex controls.
- To analyze the prevalence of numerical and structural cytogenetic aberrations and nuclear damage.
- To identify the variations in the histopathology of the testis in MTF transgenders undergoing SRS and with /or without hormonal therapy
- To analyze the RYR3 gene expression using In-Silico Mutational and RT-PCR analyses.

STUDY HYPOTHESIS

The three hypotheses used to examine the gene expression data.

Hypothesis 1:

A transsexualism can be detected by Chromosomal karyotyping

Hypothesis 2:

A histology and MN assay examination to detect the Histopathological Variation in the testis, Nuclear damage in buccal cells, useful to detect the toxicity of oestradiol conception by transgender.

Hypothesis 3:

A polymorphic gene (RYR3) gene expression analysis can be used as prognosis detection of transsexualism.

MATERIALS AND METHODS

STUDY DESIGN

- **Type of study:** Cross Sectional study
- **Study period:** 3 years
- **Departments Involved**

  Dept. of Pathology, MGMCRI

  Dept. of Surgery, MGMCRI

  Dept. of Genetics, MGMCRI
- **Study subjects:** The study involved Humans.
- **Type of Sample**: Blood sample, buccal mucosa, testis removed from Male to female transgenders undergoing sex reassignment surgery at the General surgery department, MGMCRI
- **Age group-** Above 18years to 45years.

The study was approved by the Institutional Human Ethical Committee (Project no : MGMCRI/RAC/2021/02/IHEC/60)

INCLUSION CRITERIA:

All Male to Female transgenders undergoing sex reassignment surgery at the general surgery department, MGMCRI, included in the study.

• Those who are willing to participate in the study.

• Age above 18years to 45 years only.

EXCLUSION CRITERIA:

Testis are removed due to other medical conditions are not including in the study.

SAMPLE SIZE ESTIMATION

A Minimum of 50 Male to female transgenders undergoing sex reassignment surgery at MGMCRI. Alpha (a) = 0.05 Estimated proportion , (P) = 0.0334 Estimation error (d) = 0.05. Minimum sample size needed: 50

Number of Groups

1. Cytogenetic Study
2. Micronuclei Analysis
3. Histopathological Examinations
4. Gene Expression Analysis By Real-time Quantitative

METHODOLOGY

CHROMOSOME KARYOTYPING

PATIENTS SELECTION CRITERIA

All the informed consents were introducing to our study from 2020 to 2022 with suspected gender dysphoria (GD) and Gender Reassignment Surgery were evaluated by a mental health expert (either a clinician or specialist). During the study, the patients was regularly subjected to a physical assessment (Tanner staging) and were further examined for chromosome karyotyping to analyse the chromosomal abnormalities. In total, 50 male-to-female transsexuals blood samples were collected in lithium heparin tube. All the blood samples underwent karyotype analysis.

KARYOTYPING AND STATISTICAL ANALYSIS

Molecular karyotyping was performed on blood samples by using Illumina CytoSNP/Bead Chip platform. Blood samples of 6 mL was collected from 50 Male to Female patients' transwomen. After then the collected blood samples were cultured according to standardized cytogenetic protocol described by Bates S. E. 2011. The Giemsa-banding (G-Band) staining was performed to culture the cells, and then karyotype analysis was performed on cultured

cells. Molecular karyotyping results were validated by cross-referencing known patients (Control groups - Male without GD).

MICRONUCLEI ANALYSIS

The study was approved by the Ethical Advisory Board of the Mahatma Gandhi Medical College and Research Institute before the start of sample collection and performed in accordance with the relevant guidelines and regulations. All individuals participated voluntarily, were informed about the study and informed consent was obtained from all participants.

SAMPLING, PREPARATION AND STAINING

Sampling of buccal cells was conducted with cotton sticks, which were gently rubbed 10–15× along the inner side of each cheek. After that, the stick was placed in 4 ml 0.9% saline. The buccal cells were detached from the stick with vigorous movements. This procedure was repeated once. 10 ml of buccal cell buffer (0.01 M Tris–HCl, 0.1 M EDTA, 0.02 M NaCl, 1% penicillin/streptomycin solution, pH 7.0) was added and centrifuged for 5 min at 1000 rpm. Supernatant was discarded and 5 ml buccal cell buffer added before centrifugation. These steps were repeated twice and the remaining cells were resuspended in 1 ml. Via Cytospin centrifugation, slides with 8,000 cells each were prepared. Cells were fixed in methanol at − 20 °C for at least two hours and stained with 5 µl 1:100 Gel Green (Biotium) for 30 s. Evaluation of slides was conducted with a Nikon Eclipse 55i fluorescence microscope at 400× magnification.

SCORING

The cells were scored according to the criteria previously described. All mononuclear cells appeared to be differentiated and therefore all potentially basal or differentiated cells were counted as mononuclear cells in the following. Frequencies of binuclear cells, cells with condensed chromatin and karyolytic cells were counted as well as MN frequency. Only round nuclear anomalies inside the cytoplasm but outside the nucleus with similar staining to the nucleus but only 1/16 to 1/3 the size were counted as MN. Two slides with 1,000 cells each per individual were evaluated and averaged.

HISTOPATHOLOGICAL ANALYSIS

Testis removed from MTF male to female transgenders undergoing sex Reassignment surgery. Grossing done by Pathologist followed by tissue processing . The tissue samples dissected from individuals were fixed in 10 % formalin, and processed further for paraffin section preparation. For histopathology study, a 5 μm thicknesses tissue sections were cut using a microtome (Leica RM 2025, Germany) and mounted on glass slides and stained with haematoxylin and eosin for general morphology examination

POLYMORPHIC RYR3 GENE (rs753104655) EXPRESSION ANALYSIS IN TRANS-SEXUAL INDIVIDUALS

Blood Samples Collection

50 transgenders were chosen for our experiment. 3 to 6 ml of whole blood was collected in sodium heparin tubes and Heparin, which can be sodium heparin, lithium heparin, or ammonium heparin, is present in the green tubes and acts as an anticoagulant by preventing the formation of thrombin. The collected blood samples were immediately proceeded for Total RNA Isolation.

Total RNA Isolation

The experimental procedure was similar to the one that was previously described (Zheng YM et al., 2005). Total RNAs were obtained from freshly collected whole blood in sodium heparin tubes. QIAamp RNA Blood Mini Kits was utilized to isolate total RNA from 1.5 ml of freshly collected human whole blood.

Gene Expression Analysis by Real-Time Quantitative (RT-PCR)

Using a HiMedia Insta Q96 Plus Real Time PCR Machine and a specific target gene (RYR3) forward and reverse primer with SYBR Green Super mix, cDNAs were synthesized and amplified. The primary cDNA synthesis reaction mixture contained one µl, which was used for RT-PCR PCR amplification. Depending on the linear range of PCR amplification for each gene, RT-PCR runs were typically carried out for 20 to 35 cycles. Each PCR cycle included brooding periods at 94°C for 30 s, at 55°C for 1 min, and at 72°C for 5 min. After the previous cycle, a 10 minute cycle at 72°C was run to trim polymerizations that were not completely completed. The forward and reverse primers of RYR3 gene was: 5′-AGGATATGGAGCTGGATGCC-3′, and 5′-GCACACGACAGGAGAAAGTC-3′. Endogenous control was provided by the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Each biological duplicate was used for RNA extraction and then qRT-PCR in triplicate for each of the qRT-PCR reactions. The six biological duplicates, each with a triplicate, formed the mean of the six values for the final threshold cycle (Ct). The relative amounts of each amplified product in the samples were evaluated using the comparative Ct technique. The HiMedia Insta Q96 Plus Real Time PCR System used default parameters to calculate the Ct for each reaction. Using the system's standard method, melt curve analysis of the amplified products was used to determine the qRT-PCR reactions' specificity. A negative control containing water rather than cDNA was included in each qRT-PCR reaction set. The mean values of the duplicate measurements were used in subsequent calculations after

they were averaged to determine the level of RYR3 and GAPDH gene expression in transgender patients' whole blood for the purpose of quantifying RYR3 gene expression.

RESULTS

CHROMOSOME KARYOTYPING

The role of genetics in the etiology of Gender Dysphoria is an important yet understudied area. Chromosomal investigation of Sex chromosome aberrations is involved in diagnostic management of transsexualism.

A total of 50 Male to Female patients' transgender were evaluated. Chromosomal abnormalities were performed by conventional cytogenetic study karyotyping using G-banding technique. All transgender are having normal karyotype which is 46 XY. Accidental finding was one case which had a Klinefelter variant 46 XXY. This indicates that there were no sex chromosomal disorder. Some male-to-female transgender individuals who had undergone sex reassignment surgery (SRS) showed structural chromosomal abnormalities, while others exhibited numerical abnormalities potentially linked to substance abuse, such as drugs, tobacco, and alcohol. Additionally, some patients had been administered oestrogen injections in varying dosages. A noteworthy finding was that a few cases displayed heteromorphisms of the P-stalk, which, according to literature, may be associated with infertility. Furthermore, some metaphase cells showed premature centromere division, possibly due to prolonged intake of oestradiol injections or tablets.

HISTOPATHOLOGICAL ANALYSIS

Transgenders who were undergone to the Sex Reassignment Surgery, their testis was subjected for histopathological slide preparation and we identified lot of histopathological changes observed on people with or without oestrogen therapy.

The histopathological changes were observed includes:

(1) decreased diameter of seminiferous tubules and expansion of the interstitium,

(2) marked hypoplasia of germ cells,

(3) rare cytomegaly,

(4) hypoplasia or absence of Leydig cells, and

(5) epididymal hyperplasia.

MICRONUCLEI ASSAY

The micronucleus assay of buccal mucosa cells is a straightforward biomonitoring method used to diagnose genetic damage caused by toxic agents. In the study, buccal smears were taken from a total of 50 male-to-female transgender individuals, who were then categorized into three groups: those not on any drugs, those who had taken oestrogen, and those who were on oestrogen as well as involved in substance abuse, including drugs and tobacco.

Those who were not on any drugs had normal buccal smears. However, significant variations were observed in those who had taken oestrogen, indicating the toxicity of oestrogen due to Cross Hormone Therapy. Along with that if they undergone to drug abuse, it is particular to severe which is an indicate that they may get into oral cancer. As health care providers a proper counselling in this regard would help the cause of a transgender community to preventing them from oral cancer.

GENE EXPRESSION OF RYR3 BY RT-PCR ANALYSIS

Gender dysphoria may have a genetic component, We hypothesized that gender is an innate part of identity. By utilizing quantitative real-time PCR (RT-PCR) gene expression, we analyzed the polymorphic RYR3 expression level in 50 transgender blood samples. The gene expression level of polymorphic RYR3 was analyzed statistically. Almost majority of Transgender cases showed gene expression. A significant association was identified between Gender Dysphoria and Polymorphic RYR3 expression level. Our result proposed that there is a high level of expression observed in all the groups when compared to the control group which clearly indicates the polymorphic nature of RYR 3 gene expression in the transgenders could

serve as an indicator to identify such individuals at a young age that they could suffer from Gender Dysphoria and due counselling could be served for the cause of the transgender community.

EDUCATION ON TRANSGENDER INDIVIDUALS

The education of transgender was taken up during the regular history taking , in the study identified between 18-49. It showed few had completed only primary education. The majority of the participants, had completed high school education. A few of the participants had passed higher secondary education and couple of them have also completed the undergraduate degree. Thus, the present Transgender research paved a way to a great understanding, not only personal likes or dislikes but also to make these school dropout transgenders to march forward getting degrees and further moving to higher studies paving an avenue to make them sit on professional upliftment with excellent employability.

DICSUSSION AND CONCLUSION

Gender dysphoria is most common among people undergoing cross hormone therapy and gender-affirming surgery. Therefore, studies have been conducted on chromosomal analysis using buccal smears, testicular variations, and gene expression. Karyotyping revealed that all subjects had normal chromosomal profiles, except for one accidental finding of a Klinefelter variant. This indicates that, genotypically, they are normal individuals. However, due to the toxicity from the intake of cross hormone therapy tablets or injections, we identified various structural and numerical abnormalities. These structural and numerical errors can cause significant chromosomal instability during cell division, potentially leading to numerous adverse health effects. Care should be taken to avoid long-term hormonal therapy, even if it is used for breast development. They need to undergo SRS (sex reassignment surgery) first, after which they can pursue breast development to protect against such cytogenetic abnormalities.

Also, buccal mucosa taken, the buccal cell indicated the varied kind of results In our study, we identified three groups of transgender individuals. One group did not take any cross hormone therapy (CHT) and were not smokers, and they were equivalent to the control group. However, certain individuals within this group had a history of substance abuse, which elevated their risk levels. The second group consisted of individuals who took CHT without substance abuse, showing a higher level of risk than the first group. The fourth group included individuals who both took CHT and engaged in substance abuse, exhibiting severe levels of nuclear damage. This suggests that without surgery, taking CHT along with drug use can lead to significant nuclear damage, potentially resulting in premalignant or malignant oral cancer.

Post-Gender Reassignment Surgery (GRS), we analysed testicular samples and found that individuals on CHT had varied testicular malfunctions. The age at which CHT was initiated

and the dosage taken were significant factors. We observed a high number of lumen cells, indicating that transgenders should undergo gender-affirming surgery (GAS) before starting CHT. Although this may not significantly affect the risk of testicular cancer, infertility becomes a noteworthy issue for the community. Additionally, before SRS, individuals should consider cryopreservation to preserve their family lineage for future generations.

The RYR3 gene has been found in all transgender individuals, with variations or mutations in this gene causing impulses to the brain that typically signal male gender. However, due to these mutations, the proper signals are not sent, leading individuals to feel male or female differently. This consistent finding in the transgender control group clearly indicates that RYR3 gene expression should be studied in young male-to-female transgender children. Early identification through genetic testing can lead to proper counseling, preventing future suffering for both the child and the parents.

Also, the multifaceted challenges faced by transsexual individuals in a rural tertiary care setting. While the completion of high school is a positive finding, further education and economic support are crucial for their advancement. At Sri Balaji Vidyapeeth, counselling was given to the subjects who had Sex Reassignment Surgery to continue their education. Those who passed high school were motivated to complete higher secondary school. Those who completed higher secondary school were also motivated them to pursue Higher education. As an outcome based research we identified various colleges including Govt college, Arts and Sciences, Paramedical who were ready to accept them to provide education for transgenders and also due information given about Sri Balaji Vidyapeeth, Pondicherry, India who have been offering 100% scholarship to transexuals for their education since 2019.

Thus, the present Transgender research paved a way to a great understanding, not only personal likes or dislikes but also to make these school dropout transgenders to march forward getting

degrees and further moving to higher studies paving an avenue to make them sit on professional upliftment with excellent employability.

The study clearly indicates that transgender individuals should receive proper counseling. The counselling provided in this study has been a step forward, encouraging transgender participants to inform the community about the importance of undergoing sex reassignment surgery (SRS) before starting cross hormone therapy (CHT). The toxicity of CHT can lead to severe health issues, causing numerous chromosomal and structural errors that may result in various gene-related diseases in the future. Additionally, drug abuse combined with CHT can further damage oral tissues, potentially leading to oral cancer.

The study also highlighted the significance of the RYR3 gene. Identifying variations or mutations in this gene at a young age could improve the lives of transgender individuals by facilitating early counseling, helping them continue living according to their biological sex assigned at birth if desired. Furthermore, due to testicular variations indicating infertility, it is essential for those over 18 to consider sperm preservation before undergoing such treatments. Proper counseling in all these aspects will pave the way for a better life for the transgender community at large.

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PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS

- Preetha Devi N B, & Prof Uma A N. (2024). The Impact Of Education On The Lives Of Transgender Individuals: A Comprehensive Study. *Educational Administration: Theory and Practice*, *30*(6), 3797–3800. https://doi.org/10.53555/kuey.v30i6.5425.
- Devi, P. N. B., & Uma, A. N. (2024). Significance of Micronuclei in Buccal Mucosa Smears in Transgender Undergoing Sex Reassignment Surgery. *TWIST*, *19*(2), 618-620. https://twistjournal.net/twist/article/view/445

INTERDISICIPLINARY COURSE

1. UGC course: Participated and successfully completed the UGC mandated online course on "Research and Publication Ethics" 5 weeks from 16th June, 2021 to 21st July 2021 on every week of Wednesday and Thursday from 2 Pm -5 Pm conducted by SBV.
2. Participated "UGC Mandate Webinar training on Research methodology, Bio-Statistics and preparation of scientific manuscript (RMBS)" from 18th May to 23th June 2022.

NPTEL COURSE

1. Participated and successfully completed the SWAYAM, NPTEL on the topic of "Health Research Fundamentals" 8-week course from July to September and obtained score 71%. The programme was organized by ICMR (Indian Council Medical Research) and NIE (National Institute of Epidemiology).
2. Participated and successfully completed the SWAYAM, NPTEL on the topic of "Body language :Key to professional success," 8-week course from July to September. The programme was organized by ICMR (Indian Council Medical Research) and NIE (National Institute of Epidemiology).

INTERDISCIPLINARY REFRESHER COURSE

1. Participated and successfully completed the Online interdisciplinary refresher course on the topic of " Academic Writing" from 17.08.2021 to 23.08.2021 and obtained score Grade A+. The programme was organized by Teaching Learning Centre, Ramanujan College, University of Delhi.
2. Participated and successfully completed the Online interdisciplinary refresher course on the topic of "Research Methodology" from 21.09.2021 to 05.10.2021

and obtained score Grade A+. The programme was organized by Teaching Learning Centre, Ramanujan College, University of Delhi.

POSTER PRESENTATION

1. Participated in the poster competition in National conference on “Transgender Physiology” conducted by Dhanalakshmi Srinivasan Medical College and Hospital, Perambalur on 4th September 2021.
2. Presented e poster in the topic of “MicroNuclei in Buccal Muccosa in Transgenders” organized by department of Biochemistry “Information on Reproductive Medicine” in Panimalar medical college hospital and research institute on 22.04.2022
3. Presented a poster in 4th national conference of Indian Confederation of Medical Laboratory Sciences, ICMLS’2023 entitled "Chromosomal Aberration In Transgender Undergoing Sex Reassignment Surgery” Organised by ICMLS JIPMER, Puducherry.

PAPER PRESENTATION

1. Presented the paper in the title “Histopathological Variations in Testis on Transgender Going Sex Reassignment Surgery”, on “International Webinar Convergence- World DNA DAY CONFLUENCE: EMBRACING DIVERSITY IN LAB WEEK – 2024 ”Conducted by School of Allied Health Sciences, MGMCRI, Puducherry.
2. Presented the paper at “10th International Advanced Diagnostic Techniques (ADT Symposium) – Recent trends in the diagnosis therapy in Present day health care held from 29th – 31st march, 2022 organized by Chitkara University, Punjab.